IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re application of: | Confirmation No.: 1028 |
| ZAUDERER *et al.* | Art Unit: 1639 |
| Appl. No. 10/052,942 | Examiner: Epperson, J.D. |
| Filed: January 23, 2002 | Atty. Docket: 1821.0090004/EJH/T-M |
| For: **Methods of Producing or Identifying Intrabodies in Eukaryotic Cells** | |

## Declaration Under 37 C.F.R. § 1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, the undersigned, Dr. Maurice Zauderer, residing at 44 Woodland Road, Pittsford, New York 14534, declare and state as follows:

1. I am the co-founder of Vaccinex, Inc., and have held the positions of President and Chief Executive Officer since April 6, 2001. I am also a co-inventor of the captioned patent application.

2. A current *curriculum vitae* is appended hereto as Exhibit 1.

3. I received my Ph.D. degree in cell biology from the Massachusetts Institute of Technology in 1972. From 1971 to 1975, I conducted postdoctoral research at various research institutions including the Albert Einstein College of Medicine in New York, and the National Institute for Medical Research in London. I was an Assistant Professor in the Department of Biological Sciences at Columbia University from 1976 to 1983, and from 1984 to 2000, I was an Associate Professor in the Cancer Center and Department of Microbiology and Immunology at the University of Rochester. As shown on my attached *curriculum vitae*, I have also held various other academic positions, participated in many

professional activities, and published numerous peer-reviewed articles in the field of immunology. Based on my education and experience, I am an expert in immunology and cell biology.

4. I have reviewed the above-captioned patent application ("patent application"), the final Office Action dated November 20, 2006 ("the Office Action"); U.S. Pat. No. 5,851,829 to Marasco and Haseltine ("Marasco"); Waterhouse *et al.*, *Nucleic Acids Res. 21*:2265-2266 (1993) ("Waterhouse"); PCT Publication No. WO 93/01296 to Zauderer ("Zauderer"); and PCT Publication No. WO 93/01296 to Rowlands *et al.* ("Rowlands") (collectively, "the cited references"). I have also reviewed the pending claims of the patent application.

5. The invention claimed in the patent application relates to the field of immunology. More particularly, the invention relates to the art or field of identifying, producing, and/or expressing intracellular immunoglobulins ("intrabodies") in eukaryotic cells. In my opinion, a person of ordinary skill in the art of immunology would have a Ph.D. degree in a field related to immunology or cell biology.

6. The claimed invention is directed to a screening method to select polynucleotides that encode an antigen-specific intracellular immunoglobulin molecule or fragment thereof by introducing separate libraries of immunoglobulin heavy and light chains or fragments thereof into eukaryotic host cells. Expression of the intracellular immunoglobulin induces a modified phenotype in a eukaryotic host cell by binding to an intracellular antigen.

7. It is my understanding, as explained to me by Vaccinex, Inc.'s patent attorneys, that a *prima facie* showing of obviousness of a patent claim requires that the prior

art teach each and every element of the claim, and that one of ordinary skill in the art would have been motivated to combine prior art references with a reasonable expectation of success. After reviewing Marasco and the other documents listed in paragraph 4, above, it is my opinion that the Examiner is relying on Marasco for features that it does not teach. The reasons for my opinion are set forth below.

8. The Examiner states at page 3 of the Office Action that Marasco discloses "a method of selecting polynucleotides which encode an intracellular immunoglobulin molecule or fragment thereof," and refers to excerpts of Marasco that supposedly describe screening libraries of intracellular immunoglobulins or fragments. *See* Office Action at pages 3 and 6. However, it is my opinion that the Examiner has misunderstood the teachings of Marasco.

9. Marasco's method starts either with a known antibody (see, for example, col. 13, l. 27 to col. 15, l. 27) or by identifying an antibody using conventional screening methods (see, for example, col. 12, l. 17 to col. 13, l. 26). Then, once the antibody of interest is determined, the pre-selected antibody is modified to be expressed intracellularly as an antibody fragment (see, for example, col. 15, l. 48 to col. 23, l. 12). It is also evident from the Examples section in Marasco that the specific antibodies of interest were identified and/or constructed first (Examples A and B), then were expressed intracellularly to test for activity (Example C). Thus, the purpose in Marasco was to identify an antigen-specific antibody before expressing the pre-selected antibody intracellularly for therapeutic purposes. Marasco was not identifying an immunoglobulin by screening libraries of intracellular immunoglobulins. Indeed, the specific screening methods mentioned Marasco--phage display (col. 12, ll. 3-5); passing cell culture supernatants over affinity columns, mini-gel

filtration, radioimmunoassay with magnetic beads, and biosensor-based analysis (*e.g.*, BIAcore) (col. 13, ll. 6-20); and ELISA (col. 15, ll. 38-48)--are all extracellular identification methods. None of these techniques is performed using libraries of intracellular immunoglobulins that bind an intracellular antigen. Put simply, Marasco does not teach screening libraries of intracellularly expressed immunoglobulins as the Examiner asserts. Furthermore, Marasco does not even suggest that it is possible to screen libraries of intracellularly expressed immunoglobulins, let alone provide any specific guidance to show how it would be done. Therefore, it is my opinion that that the Examiner has mischaracterized the teachings of Marasco.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,

Maurice Zauderer, Ph.D.

Date: February 8, 2007

# Curriculum Vitae

## Maurice Zauderer, Ph.D.

### Education

| | | | |
|---|---|---|---|
| Yeshiva University; NY, New York | B.S. | 1966 | Physics |
| Massachusetts Institute of Technology; Cambridge, Massachusetts | Ph.D. | 1972 | Cell Biology |

### Professional Positions:

1971-1975 Postdoctoral Fellow of the Helen Hay Whitney Foundation.
1972-1973 Postdoctoral Research with Dr. Matthew D. Scharff, Albert Einstein College of Medicine, NY.
1974-1975 Postdoctoral Research with Dr. Brigitte A. Askonas, National Institute for Medical Research, Mill Hill, London.
1975-1976 Visiting Scientist Laboratory of Cell Biology, Rome, Italy
1976-1983 Assistant Professor, Department of Biological Sciences, Columbia, University, NY, NY.
1984-2000 Associate Professor, Cancer Center and Department of Microbiology and Immunology, University of Rochester, Rochester, NY.
1984-1985 Visiting Scientist, Laboratory of Dr. Tak Mak, Ontario Cancer Institute, Toronto, Canada.
1990- 1997 Associate Professor, Strong Children's Research Center and Department of Pediatrics, University of Rochester, Rochester, New York.
1993-1994 Visiting Scientist, Laboratory of Dr. Alfred Singer, Experimental Immunology Branch, NCI, NIH, Bethesda, MD.
1997-2001 President and General Partner of Vaccinex, LP
2001- President & CEO, Vaccinex, Inc., Rochester, N.Y.

### Other Professional Activities:

1984 National Science Foundation, Cellular Physiology Study Section.
1987-1989 Associate Editor, Journal of Immunology.
1990 Allergy and Immunology Study Section, Division of Research Grants, N.I.H.
1990 National Cancer Institute Special Review Committee

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|---|---|
| 1992-1997 | Multiple Sclerosis Society, Basic Science Study Section. |
| 1994-1999 | Associate Editor, Journal of Immunology |
| 2003 - | Board Member, New York Biotechnology Association |
| 2003 - | Board Member, Rochester Economic Development Board |

**Key Scientific Publications (partial listing):**


Faherty, D.A., Johnson, D.R., and **Zauderer, M.** 1986. Origin and specificity of autoreactive T cells in antigen-induced populations. *J. Exp. Med.* 161:1293-1301.

**Zauderer, M.**, Iwamoto, A., and Mak, T. 1986. Gamma gene rearrangement and expression in autoreactive helper T cells. *J. Exp. Med.* 163:1314-1318.

Johnson, D.R., Faherty, D.A., and **Zauderer, M.** 1986. TTGG-A--L specific memory B cells induced in low responder strains. *J. Immunol.* 137:2791-2795.

Johnson, D.R., Faherty, D.A., and **Zauderer, M.** 1986. Different T cell requirements for specific memory induction in normal and xid B cells. *J. Immunol.* 137:2796-2801.

Moynihan, J., Burstyn, D., and **Zauderer, M.** 1989. Autoreactive T cell response to resting or activated B cells. *Immunol.* 68:199-203.

Burstyn, D., and **Zauderer, M.** 1989. Requirements for stimulation of autoreactive T cells by thymic stroma. *J. Immunol.* 143:1422-1425.

**Zauderer, M.** 1989. Origin and Significance of autoreactive T cells. *Advances in Immunol.* 45:417-437.

**Zauderer, M.**, and Natarajan, K. 1990. Imprint of thymic selection on autoreactive repertoires. *Immunological Reviews*. 116:159-170.

Fisher, D.J., Gigliotti, F., **Zauderer, M.** and Harmsen, A.G. 1991. Specific T-cell response to a *pneumocystis carinii* surface glycoprotein (gp120) after immunization and natural infection. *Infection and Immunity*,59: 3372.

Natarajan, K., Burstyn, D. and **Zauderer, M.** 1992. Major Histocompatibility Complex Determinants Select T-cell Receptor a Chain Variable Region Dominance in a Peptide-specific Response. *PNAS*, 89: 8874-8878.

Sahasrabudhe, D.M., Burstyn, D., Dusel, J.C., Hibner, B.L., Collins, J.L., and **Zauderer, M.** 1993. Shared T Cell-defined Antigens on Independently Derived Tumors. *J. Immunol.* 151:6302-10

Westbay, T.D., Dascher, C., Bavoil, P., and **Zauderer, M.** 1994. Dissociation of

immune determinants of outer membrane proteins of *Chlamydia psittaci* strain guinea pig inclusion conjunctivitis. *Infection and Immunity* 62:5614-23.

Westbay, T.D., Dascher, C., **Zauderer, M.**, and Bavoil, P. 1995. Deviation of immune response to *Chlamydia psittaci* outer membrane protein in LPS hyporesponsive mice. *Infection and Immunity* 63:1391-3.

**Zauderer, M.** 1996. Special delivery for peptide-stimulated immunity. *Nature Biotechnology* 14:703-705.

Moore, J.C., **Zauderer, M.**, Natarajan, K., and Jensen, P.E. 1997. Peptide binding to mixed isotype $Ab^dEa^d$ class II histocompatibility molecules. *Mol. Immunol.* 34:145-155.

**Zauderer, M.**, and Singer, A. 1997. Limiting dilution analysis of primary cytotoxic T cell precursors. *J. Immunol. Methods*, 208: 85-90.

Merchlinsky, M., Eckert, D., Smith, E., and **Zauderer,M.** 1997. Construction and characterization of Vaccinia direct ligation vectors. *Virology,* 238: 444-451.

Smith, E.S., Mandokhot, A., Evans, E.E., Mueller, L., Borrello, M.A., Sahasrabudhe, D.M., and **Zauderer, M.** 2001. Lethality-based selection of recombinant genes in mammalian cells: Application to identifying tumor antigens. *Nature Medicine*, 7:967-972.

**Zauderer Patents and Patent Applications:**

| Application Title | Filing Date |
|---|---|
| T CELLS SPECIFIC FOR TARGET ANTIGENS AND VACCINES BASED THEREON | Sept. 22, 1997 |
| METHODS FOR PRODUCING POLYNUCLEOTIDE LIBRARIES IN VACCINIA VIRUS | April 2, 2001 |
| METHODS OF SELECTING POLYNUCLEOTIDES ENCODING ANTIGENS | Jan. 3, 2002 |
| TARGETED VACCINE DELIVERY SYSTEMS | Apr. 12, 2001 |
| GENE DIFFERENTIALLY EXPRESSED IN BREAST AND BLADDER, AND ENCODED POLYPEPTIDES | Apr. 4, 2001 |
| METHODS OF PRODUCING A LIBRARY AND METHODS OF SELECTING POLYNUCLEOTIDES OF INTEREST | Mar. 28, 2001 |
| METHOD OF SCREENING FOR THERAPEUTICS FOR INFECTIOUS DISEASES | Oct. 1, 2001 |
| IN VITRO METHODS OF PRODUCING AND IDENTIFYING IMMUNOGLOBULIN MOLECULES IN EUKARYOTIC CELLS | Nov. 14, 2001 |
| METHODS OF IDENTIFYING REGULATOR MOLECULES | Feb. 4, 2002 |
| METHODS OF PRODUCING OR IDENTIFYING INTRABODIES IN EUKARYOTIC CELLS | Jan. 23, 2002 |

**Vaccinex Research Awards:**

| Date | Grant Institution | Research Area | Award Amount |
|---|---|---|---|
| 06/04 | NIH/ National Institute of Aging | Monoclonal Antibody Therapy to Combat Osteoporosis | $217,632 |
| 05/04 | National Institute Standards and Technology/ Advanced Technology Program | Development of Human Monoclonal Antibody Discovery Technology | $1,993,619 |
| 05/04 | NIH/ National Cancer Institute | New Target Antigens for Prostate Cancer Immunotherapy | $599,735 |
| 04/04 | NIH/National Institute Allergy and Infectious Diseases | Human Monoclonal Antibodies for Bioterrorism Defense | $1,358,678 |
| 02/04 | NIH/ National Cancer Institute | Functional Identification of Cancer Regulators | $363,089 |
| 03/03 | NIH/ National Cancer Institute | A Method to Identify Upstream Regulators of Oncogenes (CEA) | $433,400 |
| 02/03 | New York State Department of Labor | BUSINYS - Research Training | $16,400 |
| 05/02 | NIH/ National Institute of Aging | Genetic Selection System to Clone Osteogenic Regulators (Phase II) | $434,074 |

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|---|---|---|---|
| 09/01 | NIH/ National Institute Arthritis and Musculoskeletal and Skin Diseases | Genetic Selection to Clone Chondrogenic Regulators (Phase I) | $99,999 |
| 08/01 | NIH/ National Cancer Institute | C35: A Target for Bladder and Breast Cancer Therapy | $908,660 |
| 05/01 | New York State Department of Labor | High Tech Worker Training Program | $216,000 |
| 11/00 | National Institute Standards and Technology/ Advanced Technology Program | Cancer Antigen Identification | $2,000,000 |
| 02/00 | NIH/ National Cancer Institute | New Target Antigens for Prostate Cancer Vaccines | $477,824 |
| 07/99 | US Army Breast Cancer Research Program | Target Antigens for Breast Cancer Vaccines | $297,689 |
| | | Total | $9,416,799.00799 |

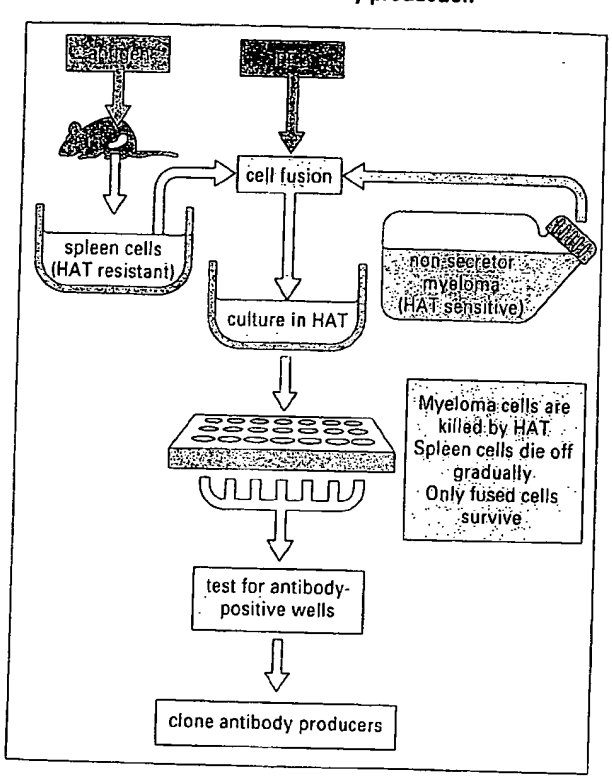


**Fig. 27.18** Animals (usually mice or rats) are immunized with antigen. Once the animals are making a good antibody response their spleens are removed and a cell suspension is prepared (lymph node cells may also be used). These cells are fused with a myeloma cell line by the addition of polyethylene glycol (PEG) which promotes membrane fusion. Only a small proportion of the cells fuse successfully. The fusion mixture is then set up in culture with medium containing 'HAT'. HAT is a mixture of hypoxanthine, aminopterin and thymidine. Aminopterin is a powerful toxin which blocks a metabolic pathway. This pathway can be bypassed if the cell is provided with the intermediate metabolites hypoxanthine and thymidine. Thus spleen cells can grow in HAT medium, but the myeloma cells die in HAT medium because they have a metabolic defect and cannot use the bypass pathway. When the culture is set up in HAT medium it contains spleen cells, myeloma cells and fused cells. The spleen cells die in culture naturally after 1–2 weeks and the myeloma cells are killed by the HAT. Fused cells survive however, as they have the immortality of the myeloma and the metabolic bypass of the spleen cells. Some of them will also have the antibody producing capacity of the spleen cells. Any wells containing growing cells are tested for the production of the desired antibody (often by solid-phase immunoassay) and if positive the cultures are cloned by plating out so that there is only one cell in each well. This produces a clone of cells derived from a single progenitor, which is both immortal and a producer of monoclonal antibody.

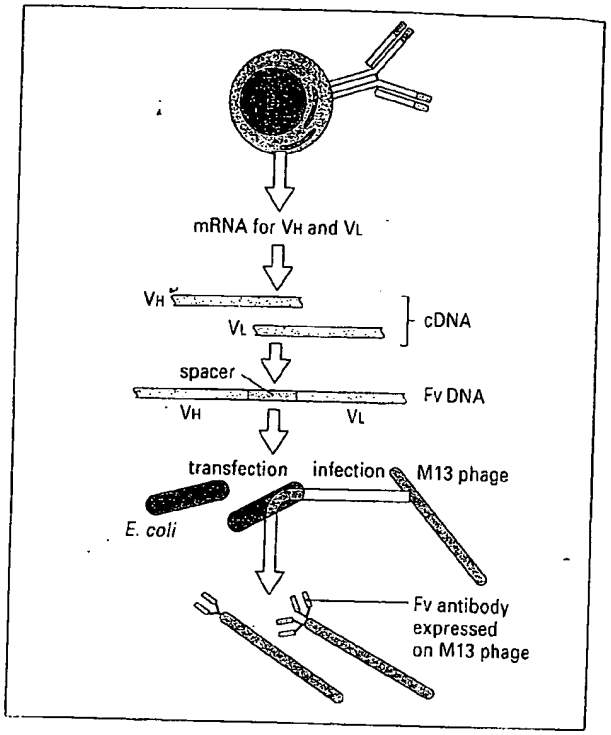


**Fig. 27.19** To produce Fv antibodies by phage display, antibody VH and VL genes are first amplified from B-cell mRNA by the polymerase chain reaction. The genes are joined together with a spacer to give a gene for an Fv fragment. Bacteria are then transfected with the gene in a phagemid vector containing a leader sequence, a fragment of the gene expressing phage coat protein 3 and an M13 origin of replication and then infected with M13 phage. The phages replicate and express the Fv on their tips. Phages displaying the right specificity are isolated by panning on antigen-coated plates and amplified. The antigen-specific phage can be used to infect strains of bacteria which allow the secretion of the Fv protein into the culture medium.

monoclonal antibody production by infecting them with Epstein–Barr virus.

A new way of generating antibodies is by phage display. In this exciting technique it is possible to express antibody-variable regions (VH and VL) as part-molecules (Fv) of defined antigen-binding specificity and affinity on the surface of M13 filamentous phage so that they can be selected by antigen. In addition, if the phages are used to infect certain bacteria, the Fv protein is secreted in large amounts into the culture medium. This approach does not necessarily require the deliberate immunization of animals or humans (*Fig. 27.19*).

Although a monoclonal antibody is a well-defined reagent it does not have a greater specificity than a polyclonal antiserum which recognizes the antigen by means of a number of different epitopes.

## ASSAYS FOR COMPLEMENT

The simplest measurement of complement activity is to determine the concentration of serum which will caus

SIXTH EDITION

# IMMUNOLOGY


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